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Morphology and Ecology of OLTMANNSIELLA VIRIDA Sp. Nov. (Chlorophyceae: Volvocales

Rhode Island Univ., Kingston

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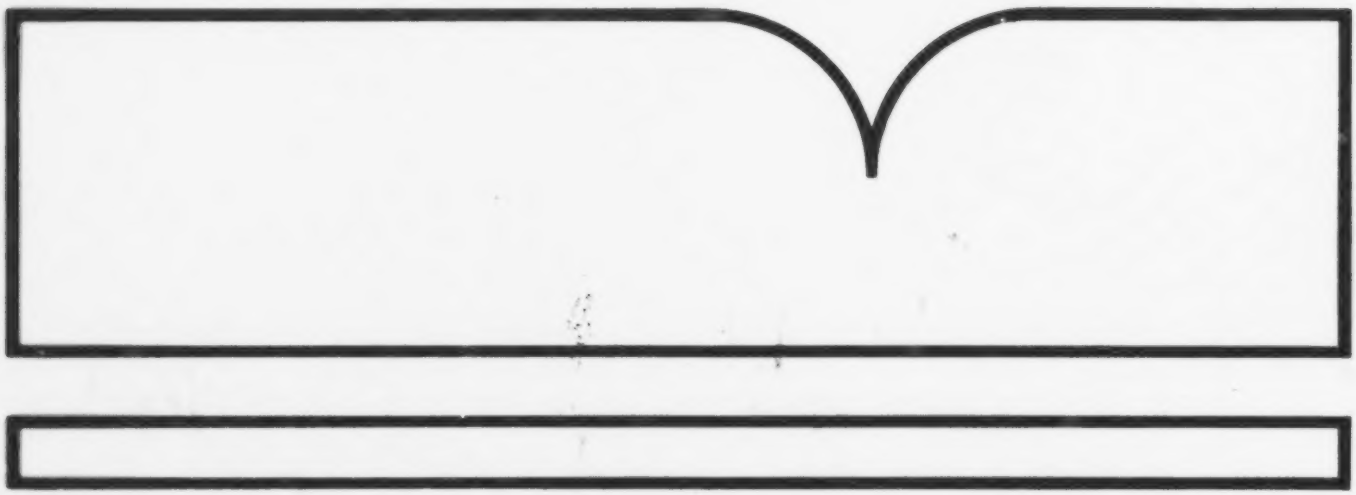

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16. ABSTRACT

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Morphology and ecology of *Oltmannsiella virida*, sp. nov. (Chlorophyceae: Volvocales)

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A new quadriflagellate green alga is described from temperate coastal waters. It occurs primarily as four-celled colonies, and its ultrastructure and general morphology place it in the Dunaliellaceae (order Volvocales, class Chlorophyceae). *Oltmannsiella virida* sp. nov. grows well in salinities from 10‰ to full-strength coastal water, and at temperatures from 15–25 C. The swimming rate of colonies varies from *c.* 225–325 μm sec^{-1} (*c.* 0·8–1·2 m h^{-1}), which is similar to other marine flagellates.

Introduction

One of the gaps in our basic knowledge of marine nanoplankton (those of about 2–20 μm in size) is the taxonomy and ecology of the organisms involved. There has been considerable interest in this group's primary productivity which may be in excess of the net plankton (20–200 μm) in both temperate (Durbin, Krawiec & Smayda, 1975; Malone, 1977) and tropical (Malone, 1971) environments. The organisms responsible are incompletely known, although many new species are described annually (*e.g.* Leadbeater, 1972; Throndsen, 1969).

During a survey of local marine plankton flagellates, a four-celled colony of a quadriflagellate green alga was isolated into culture. The organism corresponds to the genus *Oltmannsiella* established by Zimmerman (1930) from material taken in the Gulf of Naples. The type species, *Oltmannsiella lineata* Zimmerman, is a four-celled colony with two flagella per cell, a prominent posterior pyrenoid with starch sheath, posterior stigma, and a central nucleus. Zimmerman found a variation from one to eight cells per colony. Each cell was 15–22 μm long and 5–7 μm wide. During division from a unicell to a four-celled colony, the cells shed their flagella and just prior to the second division rotated 90° within a 'membrane'. Zimmerman attempted to relate this peculiar form of division to the evolution of filamentous green algae, particularly the Ulotrichales. What apparently is the same organism was subsequently found by Lackey (1962) in southern California coastal waters. Lackey's specimens were slightly larger: 20–30 μm long; 5–7 μm wide; colonies 2–7 cells, mostly four. Although Lackey found no division stages, the cells were otherwise similar to Zimmerman's. Our isolates are sufficiently different from the original description of the type species and Lackey's (1962) material to justify the establishment of a new species, which we choose to call *Oltmannsiella virida*.

Materials and methods

Oltmannsiella virida was isolated from Narragansett Bay, Rhode Island, in August 1973. The concentration of colonies in the water at the time of isolation was 5×10^3 colonies per litre. The isolation medium was 'f/2' (Guillard & Ryther, 1962). Cultures are currently maintained by both authors in Guillard's f/2 at 20 C.

Light microscopy and photomicrography were accomplished with a Zeiss Photomicroscope II with Kodak Plus-X film. For electron microscopy cells were fixed in 4% cacodylate-buffered glutaraldehyde, with 0·25 M glucose or 4% glutaraldehyde in seawater, and post-fixed in 2% osmium tetroxide, all at 4 C. The cells were dehydrated in ethanol and embedded in Spurr's low-viscosity resin (Spurr, 1969). Stained (lead citrate) and unstained diamond

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knife sections were examined in a Zeiss 9-S/2 electron microscope and photographed with Kodak 4489 film.

Temperature and salinity experiments were conducted in triplicate in 50 ml volumes in 125 ml Erlenmeyer flasks. The medium was Guillard's f/2 in Rhode Island coastal water of 30–32 $‰$, diluted as necessary for salinity tests with distilled water. Cultures were acclimatized to the various conditions before growth measurements (as changes in cell numbers) began. Cultures were incubated in a 12–$\overline{12}$ photoregime. Salinity experiments were conducted at 20 C; temperature experiments were conducted at 25$‰$ salinity. Measurements of swimming speed were made in the manner described by Throndsen (1973).

Results

Diagnosis

Cells irregularly cylindrical, apices rounded or slightly tapered, occasionally bent longitudinally; 12–30 μm long, 5–9 wide, united into four-celled linear colonies; quadriflagellate with flagella subequal to cell length and paired at an angle of 45–90 . Cell wall lacking; chloroplast single, cup-shaped; pyrenoid penetrated by thylakoids, surrounded by starch grains; stigma a posterior monolayer of lipid droplets near the plasmalemma; vegetative reproduction by longitudinal division with flagella retention; flagella and cell surface with rod-shaped particles, lacking scales or hairs.

Type locality: Narragansett Bay, Rhode Island, U.S.A.

Type illustrations: Figs 1 and 2

Type material: Clone OLT

Oltmannsiella virida sp. nov.

Cellulae irregulariter cylindricae, apicibus rotundatis aut paululum attenuatis, interdum longitudinaliter flexis, 12–30 μm long., 5–9 μm lat., in colonies linearibus quadricellularibus conjectae; cellulae quadriflagellatae, flagellis longitudine subaequis corpori et sub angulo 45–90 inter se geminatis. Membrana cellularis nulla; chloroplastus singularis, poculiformis; pyrenoides per thylakoidea penetrata, granulis amyli circumdata; stigma e monostrato posteriore guttularum liquidarum prope plasmalemma compositium; reproductio vegetativa divisione longitudinali, flagellis retentis, effecta; flagella et superficie cellulae particulis bacillaribus praedita, sine squamis pilisve.

Locus typi: Locus Narragansett Bay, Rhode Island, U.S.A. dictus

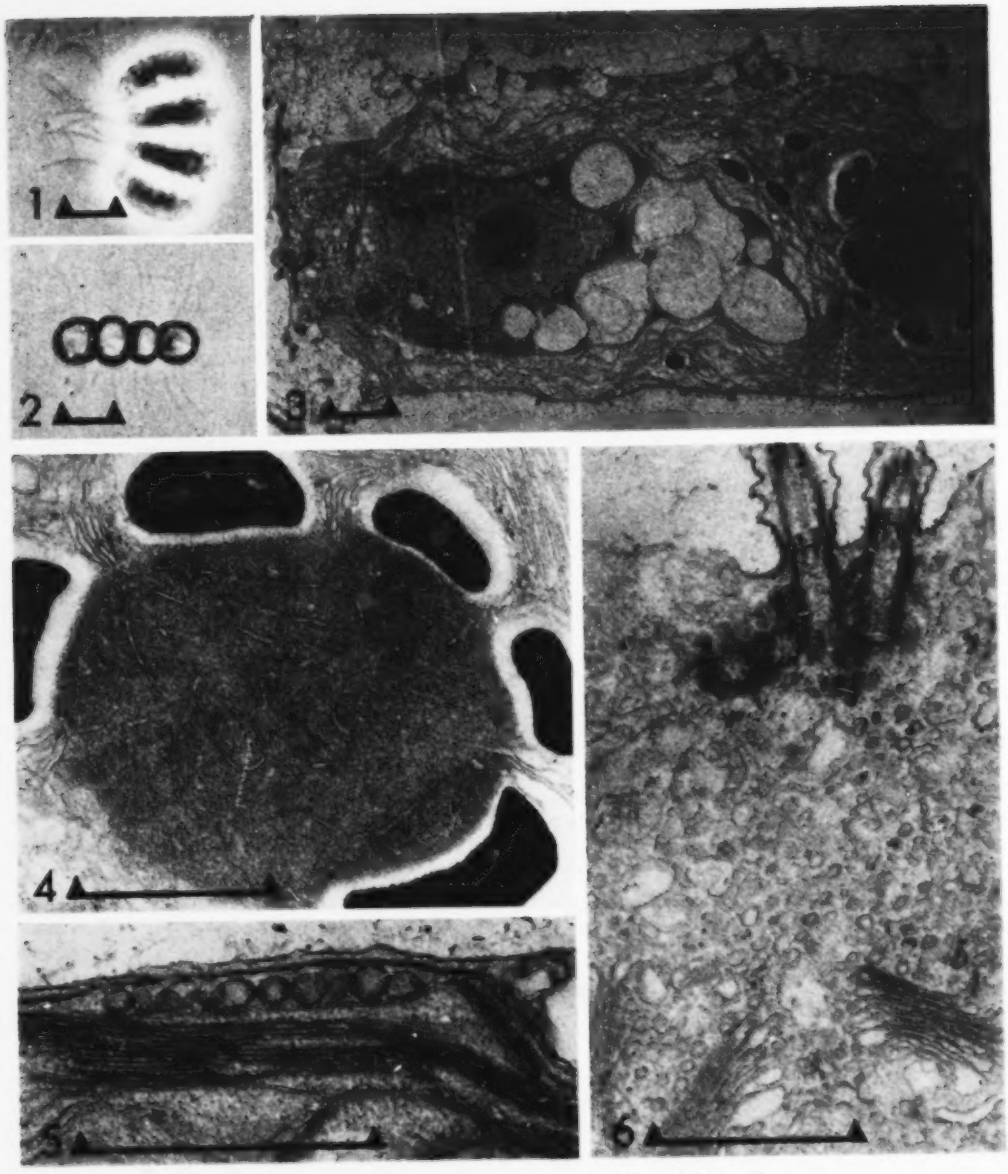
Illustrationes typi: Fig. 1 et 2

Materia typi: Clon OLT

Oltmannsiella virida is mostly a colony of four grass-green cells in a row with the cells approximately parallel in the long axis (Figs 1 and 2). In culture, colony variations from one to nine cells occur; in wild material only the four-celled colonies were seen. Nearly all the cells are quadriflagellate, although biflagellate cells which resemble *Dunaliella* appear occasionally in cultures. The flagella are approximately the same length as the cell. Individual cells in a colony are 12–30 μm in length and 5–9 μm wide. The cells are irregularly cylindrical or oval, faintly four-lobed, often with a narrowing toward the posterior. Some cells are slightly bent in the longitudinal axis. The cells are closely compacted, and the cell shape is determined by this compacting. There are no cell walls, and no polysaccharide envelope is detectable by light microscopy.

The ultrastructure of *O. virida* is similar in most respects to that of many other green flagellates. The cell lacks a wall and is bound by a membrane attached to which are a number of tiny rod-shaped particles which may be proteinaceous in nature (Dodge, 1973). These particles are usually embedded in a thin matrix exterior to the cell membrane (Fig. 7) In the area where cells adjoin, the matrix becomes somewhat more dense, and probably functions as the means by which cells in a colony are held together (Fig. 8). In the absence of confirming chemical analysis, it is reasonable to assume this matrix is polysaccharide in composition since polysaccharide is commonly and sometimes abundantly produced by green algae (see, e.g. Hellebust, 1974). There is some evidence that this matrix is released at the cell surface from vesicles, rather than exuded generally through the membrane (Fig. 3).

The flagella are paired, emerging from the cells as two pairs at an angle of 45–90 to each other (Fig. 6), which accounts for the difficulty in obtaining photomicrographs with all flagella in one plane. The structure of the flagella is what one might expect: nine doublet tubules and two central tubules; nine peripheral triplet tubules in the basal body, and a



FIGS 1–6. *Oltmannsiella virida*. Scale bars = 1 μm, except Figs 1 and 2, scale bars = 10 μm

FIG. 1. Lateral view of four-celled colony. Some flagella have been shed. Phase contrast.

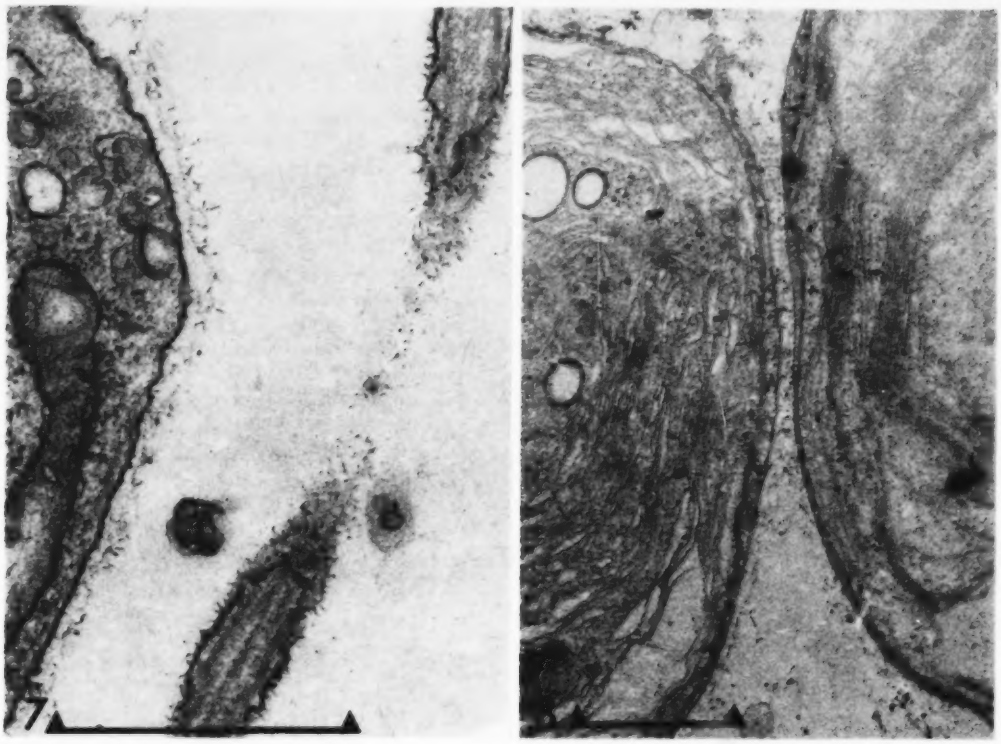
FIG. 2. Apical view of four-celled colony. Bright field.

FIG. 3. Longitudinal section of senescent cell. Arrow indicates site of release of extracellular material.

FIG. 4. Pyrenoid with starch grains and penetrating thylakoid membranes.

FIG. 5. Single-layered stigma embedded in chloroplast membrane.

FIG. 6. Apical portion of cell with four flagella bases, dictyosomes, and numerous vesicles.



FIGS 7–8 *Oltmannsiella virida*. Scale bars = 1 μm.

FIG. 7. Cell margin and tangential section of flagellum with rod-shaped particles.

FIG. 8. Junction of adjacent cells with polysaccharide matrix binding cells and lipid droplets in chloroplast.

striated root fibre system (Fig. 6). Like the cell surface, the flagella are covered with numerous rod-shaped particles; otherwise the flagella lack hairs, scales, or other appendages (Fig. 7).

The single large chloroplast has lamellae composed of several thylakoids, and is bound by the usual double membrane envelope. Lipid droplets are occasionally found scattered through the chloroplast (Fig. 8). In the posterior part of the cells a stigma is found appressed to the chloroplast membrane near the outer membrane of the cell (Fig. 5). This consists of a single layer of lipid droplets, orange-red in colour. There is no apparent connection between the stigma and the flagellar bases.

The pyrenoid (Fig. 4), is embedded in the posterior part of the chloroplast and is penetrated by sinuous chloroplast lamellae. Numerous starch grains surround the pyrenoid, the number depending somewhat on the age of the culture, and a few starch grains are occasionally found elsewhere in the chloroplast matrix (Fig. 3). The pyrenoid matrix is densely granular and probably proteinaceous in nature (Griffiths, 1970). No limiting pyrenoid membrane is apparent.

Several dictyosomes are present in the anterior portion of the cell, primarily between the nucleus and the flagella (Fig. 6). This portion of the cell is replete with small vesicles.

The facility with which *O. virida* is cultivated made possible some elementary measurements of its growth response to various fluctuations in environmental conditions. Responses to salinity were examined in dilutions of Rhode Island coastal water (Fig. 9). Growth was rather uniform in salinites from 15–30‰ (about 0·5–0·6 divisions per day). At 10‰, growth was less and in the lowest salinities cell death

occurred. The slope of the growth curves is slightly different between 15 and 30‰, but since each data point represents a mean, there is no reason to attach any significance to these minor differences.

In response to temperature (Fig. 10) *O. virida* grew best at 25 C and progressively less successfully at temperatures down to 5 C. The rapid die-off after 11 days at 25 C is probably due to combined effects of nutrient depletion and self-shading, since these cultures were grown in closed systems rather than chemostats. The growth rate varies from over 0·6 div/day at 25 C to less than 0·1 div/day at 5 C. The temperature and salinity data compare rather favourably with the known occurrence of the species in local waters. *O. virida* was found only in the warmer months (temperature 20 C or greater) and at coastal salinities of about 20–30 ‰.

Swimming was measured by recording swimming tracks photographically and comparing them with measurements of various other marine flagellates as a function of volume (Throndsen, 1973). *O. virida* colonies, positively phototactic, swim at a rate of 225–325 ($\bar{x}=290$) μmsec^{-1}, or about 0·8–1·1 m h^{-1} (Fig. 11).

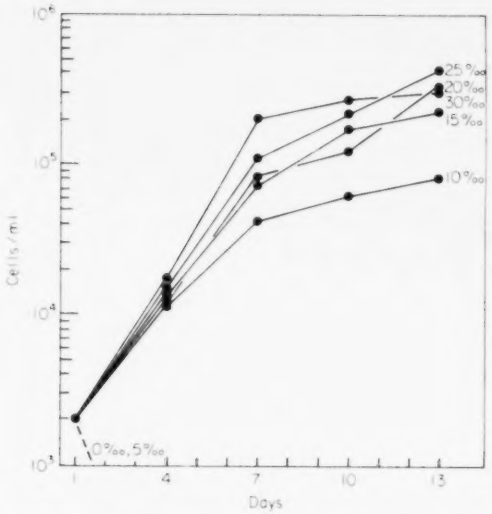


FIG. 9. *O. virida*, growth response to salinity. Each data point is mean of three replicates.

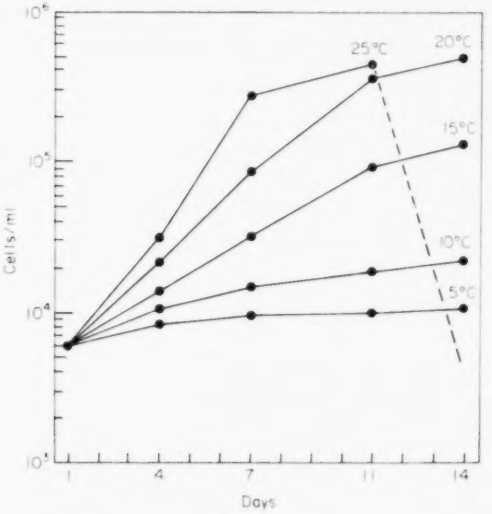


FIG. 10. *O. virida*, growth response to temperature. Each data point is mean of three replicates.

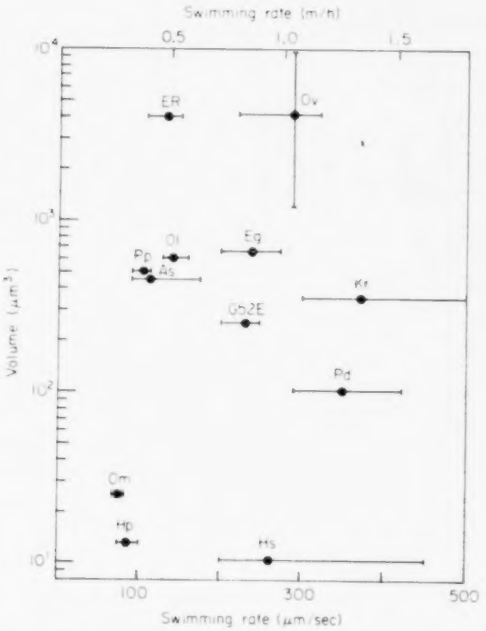


FIG. 11. *O. virida*, swimming rate and colony volume range compared with swimming rate and volume of other marine flagellates, from Throndsen (1973). (Ov = *O. virida*; ER = *Eutreptiella* sp. R; Eg = *Eutreptiella gymnastica* As = *Apedinella spinifera*; Kr = *Katodinium rotundatum*; G52E = *Gyrodinium* sp. 52E; Pd = *Pyramimonas* cf. *disomata*; Om = *Ochromonas minima*; Hp = *Heteromastex pyriformis*; *Hs* = *Hemiselmis simplex*.

Compared with the measurements made by Throndsen, there is nothing particularly unusual in the swimming rate of *O. virida*. This graphical array emphasizes Throndsen's observation that, despite a size variation of about three orders of magnitude, the variation in swimming rate is relatively small.

Discussion

O. virida differs from *O. lineata* primarily in the presence of four flagella rather than two. This difference alone is considered sufficient to establish different genera by some authors (e.g. *Carteria* vs *Chlamydomonas*; *Spondylomorum* vs *Pyrobotrys*), but establishing a new genus would detract from the obvious similarities of *O. virida* to *O. lineata*. Other green flagellate genera which have been grouped in the Volvocales such as *Pyramimonas* (which is alternatively included in a separate class, Prasinophyceae, by many authors) contains species with eight or four (sometimes two?) flagella. Likewise *Polyblepharides* may have six or eight flagella. The occasional presence of biflagellate cells (only in culture) may imply the existence of life-cycle stages, as yet undescribed, relating the two taxa more closely. Differences between *O. virida* and *O. lineata* in the mode of cell division (in *O. virida*, flagella present through division, surrounding membrane is lacking, thus no rotational movement during division) represent basic genetic differences, and perhaps these alone justify separating the two taxa.

At the generic level, several other quadriflagellate green volvocalean flagellates are known (excluding prasinophytes). *Quadrichloris* Fott is unicellular and confined to alpine pools; *Collodictyon* Carter is lobed and apochlorotic; and *Polytomella* Aragao is apochlorotic and normally has a distinct apical papilla. *Oltmannsiella* is sufficiently distinct from all of these [see Huber-Pestalozzi (1961) for full generic descriptions].

Spermatazopsis, a freshwater genus, has some features in common with *Oltmannsiella*. Most described species are normally quadriflagellate, with flagella persisting through division (Belcher, 1964). Korschikov (1913) found (as in *O. virida*) both quadri- and biflagellate forms in *S. exsultans*. Pascher (1927) considered these biflagellates as the normal condition, with quadriflagellates as holozygotes. Kalina (1965) describes *S. acidophila* as biflagellate, but Bourrelly (1966) and Heynig (1969) consider all these biflagellate forms as representing a different genus. *O. virida* is quadriflagellate in nature, but biflagellates occur in clonal cultures. These may represent as yet undescribed life cycle stages. *Spermatozopsis* differs from *Oltmannsiella* in being unicellular, having fusiform or spindle-shaped cells, forming cysts, and being exclusively a freshwater genus. At the ultrastructural level, *Spermatozopsis* flagella have 'hair-points' (Manton, 1965) which *O. virida* flagella lack, while the flagellar rod-shaped particles (resembling the flagellar scales of *Pseudoscourfieldia marina* (Manton, 1975) and *Astermonas gracilis* Artari (Peterfi & Manton, 1968)) are lacking in *Spermatozopsis*.

Two other genera deserve brief mention; namely, *Raciborskiella* Wislouch and *Korschikoffia* Pascher. The former is clearly different from our organism (Huber-Pestalozzi, 1961). *Raciborskiella*, while forming colonies, is strictly biflagellate. The cells are egg-shaped or antapically attenuated. The colonies, up to sixteen radially arranged cells, are united only at the anatapical ends. *Korschikoffia* is a biflagellate unicellular genus. The cell is basically egg-shaped with an elongate apex, but strongly metabolic (Bourrelly, 1966; Huber-Pestalozzi, 1961); the chloroplast is found in the lower part of the cell; and cysts are formed under adverse conditions.

The above discussion deals with comparison of our new species, *O. virida* with related genera. Yet to be established (or examined by modern techniques) are the relationships among the quadriflagellate *O. virida*, the biflagellate *O. lineata*, and the other genera mentioned. In all likelihood some of these genera, conceivably even *Oltmannsiella*, may eventually be synonymized when ultrastructural details, life cycles, and morphological variations are known, but such is beyond the scope of this report.

The absence of a cell wall, longitudinal division, and details of morphology in the light and electron microscopes compare sufficiently well to place our organism in close proximity to *Dunaliella* (Eyden, 1975; Levain & Marano-Le Baron, 1973; Peterfi & Manton, 1968; Trezzi, Galli & Bellini, 1964) in the family Dunaliellaceae as used by Parke & Dixon (1976). The variations observed in culture (1–8 cells, biflagellate cells, morphological teratologies, etc.) are probably a consequence of stress induced by growth under abnormal conditions but may also represent life cycle stages. Wild material was uniformly found as four-celled colonies with quadriflagellate cells. The limitation of colony size to four cells under natural conditions is conceivably a

consequence of water viscosity resistance and turbulence, in light of the seemingly weak nature of the material joining cells together. Still unexplained is the mechanism by which flagella beat and stop in apparent unison with a colony in the absence of intercellular connection other than the presumed polysaccharide exudate.

The growth characteristics of *O. virida* indicate its adaption to a coastal temperature environment. Despite 20 years of phytoplankton observation in the area (Hitchcock & Smayda, 1977), *O. virida* has not been previously recorded. It may have been a recent invader of this area from contiguous waters, or it may have been present for some time in numbers low enough to escape notice. Considering the intensity with which phytoplankton research has been pursued in several laboratories in New England, it is the latter alternative which is more likely. A similar case has been noted for the ebridian flagellate *Hermesinum adriaticum* Zach. (Hargraves & Miller, 1974). The sudden relative abundance of *O. virida* was most probably a consequence of a peculiar set of environmental circumstances which permitted its success in local waters during 1973. Since that time it has been recorded during summer months, but not in the numbers (5×10^3 colonies/litre) first noted.

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